

WHAT IS CLAIMED IS:

- 1 1. A composition comprising a peptide that binds to six or more different
2 members of a panel of HLA-DR molecules, wherein the peptide comprises at least about
3 nine amino acids, said peptide having the formula $Z_n-X_1X_2X_3X_4X_5X_6X_7X_8X_9-Z_c$, wherein:
4 X_1 is an amino acid selected from the group consisting of: (X), Y, F, M,
5 L, I, V, and W, wherein (X) is cyclohexylalanine;
6 X_2 is an amino acid selected from the group consisting of: I and V;
7 X_3 , X_4 , X_5 , X_7 , X_8 , and X_9 are each an amino acid;
8 X_6 is an amino acid selected from the group consisting of: T, V, M, S,
9 A, C, P, L, and I;
10 Z_n and Z_c each comprise 1 to about 15 amino acids.
- 1 2. The composition of claim 1, wherein either or both of Z_n and Z_c
2 comprise at least one D-amino acid.
- 1 3. The composition of claim 1, wherein Z_n comprises K or A adjacent to
2 X_1 ; X_1 is (X), Y or F; and X_2 is I or V.
- 1 4. The composition of claim 1, wherein the peptide inhibits activation of a
2 T cell which displays a receptor which is specific for a DR molecule and X_5 is a non-polar,
3 non-charged, non-bulky, non-aromatic amino acid.
- 1 5. The composition of claim 4, wherein X_5 is A.
- 1 6. The composition of claim 4, wherein one or more of X_3 , X_4 , X_7 , X_8 , and
2 X_9 is a non-polar, non-charged, non-bulky, non-aromatic amino acid.
- 1 7. The composition of claim 6, wherein Z_n comprises at least one non-
2 polar, non-charged, non-bulky, non-aromatic amino acid.

1 8. The composition of claim 6, wherein one or more of X₃, X₄, X₇, X₈, and
2 X₉ is A.

1 9. The composition of claim 1, wherein the peptide stimulates activation of
2 a T cell which displays a receptor specific for a DR molecule and wherein X₅ is a polar,
3 charged, bulky, or aromatic amino acid.

1 10. The composition of claim 9, wherein X₅ is W.

1 11. The composition of claim 9, wherein one or more of X₃, X₄, X₇, X₈, and
2 X₉ is a polar, charged, bulky, or aromatic amino acid.

1 12. The composition of claim 11, wherein one or more of X₃, X₄, X₇, X₈,
2 and X₉ is W.

1 13. A composition comprising a peptide that binds to six or more different
2 members of a panel of HLA-DR molecules, wherein the peptide comprises at least about
3 nine amino acids, said peptide having the formula Z_n-X₀X₁X₂X₃X₄X₅X₆X₇X₈X₉-Z_c,
4 wherein:

5 X₀ is an amino acid selected from the group consisting of: K and A;

6 X₁ is an amino acid selected from the group consisting of: (X), Y and F;

7 X₂ is an amino acid selected from the group consisting of: I and V;

8 X₃, X₄, X₅, X₇, X₈, and X₉ are each an amino acid;

9 X₆ is T;

10 Z_n and Z_c each comprise 1 to about 15 amino acids.

1 14. The composition of claim 13, wherein either or both of Z_n and Z_c
2 comprise at least one D-amino acid.

1 15. The composition of claim 13, wherein the peptide inhibits activation of
2 a T cell which displays a receptor specific for a DR molecule and wherein X₅ is a non-polar,
3 non-charged, non-bulky, non-aromatic amino acid.

1 16. The composition of claim 15, wherein X₅ is A.

1 17. The composition of claim 15, wherein one or more of X₃, X₄, X₇, X₈
2 and X₉ is a non-polar, non-charged, non-bulky, non-aromatic amino acid.

1 18. The composition of claim 15, wherein either or both of Z_n and Z_c
2 comprises at least one non-polar, non-charged, non-bulky, non-aromatic amino acid.

1 19. The composition of claim 13, wherein the peptide stimulates activation
2 of a T cell which displays a receptor specific for a DR molecule and wherein X₅ is a polar,
3 charged, bulky, or aromatic amino acid.

1 20. The composition of claim 19, wherein X₅ is W.

1 21. The composition of claim 19, wherein one or more of X₃, X₄, X₇, X₈,
2 and X₉ is a polar, charged, bulky, or aromatic amino acid.

1 22. The composition of claim 21, wherein one or more of X₃, X₄, X₇, X₈,
2 and X₉ is W or K.

1 23. The composition of claim 22, wherein one or more of X₇ and X₈ is K.

1 24. The composition of claim 1, wherein the panel of HLA-DR molecules
2 comprises at least six members selected from the group consisting of DR1, DR2w2b,
3 DR2w2a, DR3, DR4w4, DR4w14, DR5, DR6, DR7, DR8, DR9, DR52a, DR52b, DR52c
4 and DR53.

1 25. The composition of claim 1, wherein the peptide is a pan-DR peptide
2 that binds to at least about seven members of the panel with high affinity.

1 26. The composition of claim 1, wherein the composition comprises a
2 pharmaceutically acceptable carrier.

1 27. The composition of claim 1, which further comprises a CTL inducing
2 peptide.

1 28. The composition of claim 27, wherein the CTL inducing peptide is
2 acetylated, palmitylated, or acylated with a fatty acid.

1 29. The composition of claim 27, wherein the CTL inducing peptide is
2 linked to the peptide to form a CTL/T helper peptide conjugate.

1 30. The composition of claim 29, wherein the CTL/T helper peptide
2 conjugate is linked to a carrier.

1 31. The composition of claim 29, wherein the CTL inducing peptide is
2 linked to the peptide by a spacer molecule.

1 32. The composition of claim 1, wherein the peptide is a naturally occurring
2 peptide that arises upon processing of a protein by an antigen-presenting cell.

1 33. The composition of claim 32, wherein the peptide is a human peptide.

1 34. The composition of claim 1, wherein the peptide is linked to one or
2 more carbohydrate epitopes and the composition can induce an immune response to an
3 antigenic carbohydrate.

1 35. The composition of claim 34, wherein the carbohydrate epitope
2 comprises an antigenic determinant from a bacterium, a virus, a cancer cell, a fungus, or a
3 parasite.

1 36. The composition of claim 34, wherein the carbohydrate epitope is
2 linked to the carboxy-terminus of the peptide.

1 37. The composition of claim 34, wherein the carbohydrate epitope is
2 covalently linked to the PADRE peptide through a linker.

1 38. The composition of claim 37, wherein the linker comprises a cysteine
2 residue.

1 39. The composition of claim 37, wherein the linker consists of an
2 aminocaproic acid residue and a cysteine residue.

1 40. The composition of claim 34, wherein the peptide is further linked to a
2 surface-active material.

1 41. The composition of claim 40, wherein the surface-active material is a
2 lipid moiety, a polymer, polyalkylene glycol, or a surfactant.

1 42. The composition of claim 40, wherein the surface-active material is
2 linked to the N-terminus of the PADRE peptide.

1 43. The composition of claim 40, wherein the surface-active material
2 comprises palmitic acid.

1 44. The composition of claim 43, wherein the surface-active material is a
2 lipid moiety which is PAM₂K, wherein K is a lysine residue and PAM is a palmitic acid
3 residue.

1 **45.** A peptide that binds to seven or more members of a panel of HLA-DR
2 molecules, the peptide comprising a neutral amino acid at each position that is not a critical
3 contact site, which critical contact sites are necessary for binding of the antigen to a selected
4 HLA-DR molecule.

1 **46.** A method for identifying a pan-DR peptide, the method comprising
2 analyzing one or more peptide sequences for a peptide that has the formula Z_n -
3 $X_1X_2X_3X_4X_5X_6X_7X_8X_9-Z_c$, wherein:
4 X_1 is an amino acid selected from the group consisting of: (X), Y, F, M,
5 L, I, V, and W, wherein (X) is cyclohexylalanine;
6 X_2 is an amino acid selected from the group consisting of: I and V;
7 X_3 , X_4 , X_5 , X_7 , X_8 , and X_9 are each an amino acid;
8 X_6 is an amino acid selected from the group consisting of: T, V, M, S,
9 A, C, P, L, and I;
10 Z_n and Z_c each comprise 1 to about 15 amino acids; and
11 selecting a peptide or peptides that have such formula.
12

1 **47.** The method of claim **46**, further comprising the step of testing the
2 selected peptide or peptides for binding to three different members of a panel of HLA-DR
3 molecules that comprises at least three members selected from the group consisting of DR1,
4 DR2w2b, DR2w2a, DR3, DR4w4, DR4w14, DR5, DR6, DR7, DR8, DR9, DR52a, DR52b,
5 DR52c, and DR53.

1 **48.** The method of claim **46**, wherein the selected peptide is a naturally
2 occurring peptide.

1 **49.** The method of claim **46**, wherein the peptides are tested for binding to
2 sequential panels of HLA-DR molecules.

1 50. The method of claim 49, wherein the sequential panel comprises a
2 primary panel which comprises DR1, DR4w4 and DR7 HLA molecules.

1 51. The method of claim 50, wherein peptides that bind to at least two
2 members of the primary panel are tested for binding to members of a secondary panel which
3 comprises DR2w2 β 1, DR2w2 β 2, DR6w19 and DR9 HLA molecules.

1 52. The method of claim 51, wherein peptides that bind to at least three
2 members of the secondary panel are tested for binding to members of a tertiary panel which
3 comprises DR4w15, DR5w11 and DR8w2 HLA molecules.

1 53. A method for rational design of a peptide that binds to three or more
2 different members of a panel of HLA-DR molecules, which peptide has the formula Z_n-
3 X₁X₂X₃X₄X₅X₆X₇X₈X₉-Z_c, the method comprising:

4 introducing at position X₁ an amino acid selected from the group
5 consisting of (X), Y, F, M, L, I, V, and W, wherein (X) is cyclohexylalanine;

6 introducing at position X₂ an amino acid selected from the group
7 consisting of I and V; and

8 introducing at position X₆ an amino acid selected from the group
9 consisting of T, V, M, S, A, C, P, L, and I;

10 wherein X₃, X₄, X₅, X₇, X₈, and X₉ are each an amino acid; and

11 Z_n and Z_c each comprise 1 to about 15 amino acids.

1 54. The method of claim 53, wherein the method further comprises testing
2 the peptide to identify those that bind with high to intermediate affinity to three or more
3 different members of a panel of HLA-DR molecules.

1 55. The method of claim 53, wherein the panel of HLA-DR molecules
2 comprises at least three members selected from the group consisting of DR1, DR2w2b,
3 DR2w2a, DR3, DR4w4, DR4w14, DR5, DR6, DR7, DR8, DR9, DR52a, DR52b, DR52c,
4 and DR53.

1 **56.** The method of claim **53**, wherein the peptide is a pan-DR peptide that
2 binds to at least about three members of the panel with high affinity.

1 **57.** The method of claim **56**, wherein the pan-DR peptide binds to at least
2 about three members of the panel with an IC_{50} of about 500 nM or less relative to the IC_{50} of
3 a reference peptide.

1 **58.** The method of claim **53**, wherein the method further comprises
2 rationally designing the peptide to inhibit DR-restricted T cell proliferation by replacing one
3 or more amino acids at positions X_3 , X_4 , X_5 , X_7 , X_8 , and X_9 with a non-polar, non-charged,
4 non-aromatic, non-bulky amino acid.

1 **59.** The method of claim **58**, wherein the non-polar, non-charged, non-
2 aromatic, non-bulky amino acid is selected from the group consisting of Ala, Gly, and Pro.

1 **60.** The method of claim **59**, wherein the non-polar, non-charged, non-
2 aromatic, non-bulky amino acid is Ala.

1 **61.** The method of claim **58**, wherein each amino acid at positions X_3 , X_4 ,
2 X_5 , X_7 , X_8 , and X_9 is replaced with a non-polar, non-charged, non-aromatic, non-bulky
3 amino acid.

1 **62.** The method of claim **58**, wherein the method further comprises:
2 identifying those test peptides that retain their ability to bind with high
3 to intermediate affinity to greater than 50% of members of a panel of HLA-DR molecules;
4 and
5 testing the peptides that retain HLA-DR binding ability to identify those
6 that can inhibit DR-restricted T cell proliferation.

1 63. The method of claim 62, wherein the testing of the peptides to identify
2 those that can inhibit DR-restricted T cell proliferation is conducted in an *in vitro* assay
3 system or in an *in vivo* system.

1 64. A peptide that binds to greater than 50% of members of a panel of
2 HLA-DR molecules, wherein the peptide is identified using the method of claim 53.

1 65. The method of claim 53, wherein the method further comprises
2 rationally designing the peptide to induce an immune response by including at one or more
3 of positions X₃, X₄, X₅, X₇, X₈, and X₉ a polar, charged, aromatic, or bulky amino acid.

1 66. The method of claim 65, wherein the polar, charged, aromatic or bulky
2 amino acid is selected from the group consisting of W, K, and (X).

1 67. The method of claim 65, wherein positions X₇ and X₈ are each
2 independently selected from the group consisting of W and K.

1 68. The method of claim 65, wherein the method further comprises:
2 identifying those test peptides that retain their ability to bind with high
3 to intermediate affinity to greater than 50% of members of a panel of HLA-DR molecules;
4 and
5 testing the pan-DR peptides that retain HLA-DR binding ability to
6 identify those that can induce an immune response.

1 69. The method of claim 68, wherein the amino acid is selected from the
2 group consisting of cyclohexylalanine, tryptophan, and lysine.

1 70. The method of claim 68, wherein the immune response is a humoral
2 response or a cytotoxic response.

1 71. The method of claim 68, wherein the peptides are tested *in vivo*.

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1 72. A peptide that comprises a pan-DR T helper epitope that binds to three
2 or more different members of a panel of HLA-DR molecules, wherein the peptide is
3 obtained using the method of claim 68.

1 73. A method of inhibiting antigen-specific activation of T cells, the method
2 comprising contacting a composition that comprises antigen presenting cells and the T cells
3 with a peptide that binds to three or more different members of a panel of HLA-DR
4 molecules, wherein:

5 the antigen presenting cells display a member of the panel of HLA-DR
6 molecules and the T cells are of the DR MHC restriction; and

7 at least one amino acid residue in the peptide which is not a critical
8 contact site for binding to the HLA-DR molecule but is involved in binding to a T cell
9 receptor is substituted with a neutral amino acid.

1 74. The method of claim 73, wherein the method comprises administering
2 to a patient a therapeutically effective dose of a pharmaceutical composition that comprises
3 the peptide and a pharmaceutically acceptable carrier.

1 75. A method of inducing antigen-specific activation of T cells, the method
2 comprising contacting a composition that comprises antigen presenting cells and the T cells
3 with a peptide that binds to three or more members of a panel of HLA-DR molecules,
4 wherein the antigen presenting cells display a member of the panel of HLA-DR molecules
5 and the T cells are of the DR MHC restriction, and further wherein the peptide has at least
6 one characteristic selected from the group consisting of:

7 at least one amino acid which does not constitute a critical contact site
8 in a pan-DR peptide from which the peptide is derived is substituted with an amino acid
9 having a side chain that has one or more properties selected from the group consisting of
10 increased bulk, hydrophobicity, aromaticity and charge compared to the substituted amino
11 acid;

12 the peptide is conjugated to a CTL-inducing antigenic determinant; and

13 the peptide is conjugated to a humoral response-inducing antigenic
14 determinant.

1 **76.** The method of claim **75**, wherein the CTL-inducing antigenic
2 determinant or the humoral response-inducing antigenic determinant comprises a
3 carbohydrate epitope.

1 77. An isolated nucleic acid molecule that encodes a peptide of claim 1.

Add a 1

Figure 1 consists of 15 subplots, each representing a different value of k from 0 to 14. Each subplot is a histogram showing the frequency of the number of non-zero elements in the rows of the matrix A_k . The x-axis for all plots is 'Number of non-zero elements' with major ticks at 0, 20, 40, 60, 80, and 100. The y-axis is 'Frequency' with major ticks at 0, 2, 4, 6, 8, and 10. The distributions are generally unimodal and shift towards higher numbers of non-zero elements as k increases. For $k=0$, the distribution is centered around 10-20. For $k=14$, the distribution is centered around 60-70.

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